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Karyotypes of central European spiders of the genera *Arctosa*, *Tricca*, and *Xerolycosa* (Araneae: Lycosidae)

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Abstract. The aim of this study was to characterize karyotypes of central European spiders of the genera *Arctosa*, *Tricca*, and *Xerolycosa* (Lycosidae) with respect to the diploid chromosome number, chromosome morphology, and sex chromosomes. Karyotype data are reported for eleven species, six of them for the first time. For selected species the pattern in the distributions of the constitutive heterochromatin and the nucleolar organizer regions (NORs) was determined. The silver staining technique for detecting NORs of lycosid spiders was standardized. The male karyotype consisted of 2n = 28 (*Arctosa* and *Tricca*) or 2n = 22 (*Xerolycosa*) acrocentric chromosomes. The sex chromosome system was X_1X_20 in all species. The sex chromosomes of *T. lutetiana* and *X. nemoralis* showed unusual behaviour during late diplotene, namely temporary extension due to decondensation. C-banding technique revealed a small amount of constitutive heterochromatin at the centromeric region of the chromosomes. Two pairs of autosomes bore terminal NORs. Differences in karyotypes among *Arctosa* species indicate that the evolution of the karyotype in this genus involved autosome translocations and size changes in the sex chromosomes. Based on published results and those recorded in this study it is suggested that the ancestral male karyotype of the superfamily Lycosoidea consisted of 28 acrocentric chromosomes.

INTRODUCTION

The family Lycosidae (wolf spiders) belongs to the superfamily Lycosoidea that is included in the entelegyne lineage of araneomorph spiders (Jocqué & Dippenaar-Schoeman, 2007). The lycosids occur all over the world and are very diverse with 2367 species in 116 genera described so far (Platnick, 2010). The present study concentrated on three lycosid genera, namely *Arctosa* C.L. Koch, 1847, *Tricca* Simon, 1889, and *Xerolycosa* Dahl, 1908. Eight species and one subspecies of the genus *Arctosa*, one species of the genus *Tricca*, and two species of the genus *Xerolycosa* occur in central Europe (Blick et al., 2004). All were included in this study except *A. stigmosa* (Thorell, 1875), which is very rare.

Each of the species investigated has a different biology. However, they can be placed into three ecological groups based on their habitat preferences (Buchar & Thaler, 1995; Buchar & Růžicka, 2002): Five species prefer dry habitats – *A. figurata* (Simon, 1876), *A. perita* (Latreille, 1799), *T. lutetiana* (Simon, 1876), *X. miniata* (C.L. Koch, 1834), and *X. nemoralis* (Westring, 1861). Four species occur in humid habitats – *A. alpigena lamperti* Dahl, 1908, *A. cinerea* (Fabricius, 1777), *A. leopardus* (Sundevall, 1833), and *A. maculata* (Hahn, 1822). The remaining two are alpine species – *A. alpigena* (Doleschall, 1852) and *A. renidescens* Buchar & Thaler, 1995.

The genera *Arctosa* and *Tricca* are closely related (Braun, 1963; Buchar, 1981) and placed by Petrunkevitch (1928) in the subfamily Lycosinae, whereas according to Zyuzin (1985) *Xerolycosa* belongs to the subfamily Evippinae. The relationships within the genus *Arctosa* remain to be resolved. Today, the genus *Arctosa* contains species placed formerly in separate genera and species of doubtful taxonomical position. The latter is the case for several central European species, namely the *alpigena*-group [*A. alpigena*, *A. a. lamperti*, *A. renidescens*, and *A. insignita* (Thorell, 1872)] and *A. figurata*. Simon (1937) delimited the *alpigena*-group as a separate group within the genus *Lycosa* Latreille, 1804. Remarkably, most of the other *Lycosa* groups delimited in his study have currently a generic status. Concerning *A. figurata*, Zyuzin (1985) states that because of differences in the structure of its genitalia this species does not belong to the genus *Arctosa*. The synonymization of the genus *Tricca* with the genus *Arctosa* (Platnick, 2010) is not accepted here. Based on morphological characters differentiating both genera, the genus *Tricca* will be revalidated (Y.M. Marusik, P.T. Lehtinen & P. Dolejš, unpubl.). Unlike the genus *Arctosa* the genus *Xerolycosa* is a well defined group containing only five species (Platnick, 2010).

In terms of cytogenetics, lycosids are one of the best explored families of entelegyne spiders along with the families Araneidae and Salticidae. Despite this, there are many lycosid genera for which the karyotypes are

TABLE 1. Basic karyotypes of the genera *Arctosa*, *Tricca*, and *Xerolycosa*.

Species	2n	n (male)	References
Arctosa alpigena (Doleschall, 1852)	26 (♂)	12+X_1X_2	Hackman, 1948
	30 (♀)		this study
Arctosa alpigena lamperti Dahl, 1908	28 (♂)	13+X_1X_2	this study
Arctosa cinerea (Fabricius, 1777)	28 (♂)	13+X_1X_2	this study
Arctosa figurata (Simon, 1876)	28 (♂)	13+X_1X_2	this study
Arctosa leopardus (Sundevall, 1833)	26 (♂)	12+X_1X_2	Hackman, 1948
	28 (♂)	13+X_1X_2	this study
Arctosa maculata (Hahn, 1822)	28 (♂)	13+X_1X_2	this study
Arctosa mulani (Dyal, 1935)	28 (♂)	13+X_1X_2	Sharma et al., 1958 (sub. *Pardosa mulani*)
Arctosa perita (Latreille, 1799)	12 (♀)		Akan et al., 2005
	28 (♂)	13+X_1X_2	this study
Arctosa renidescens Buchar & Thaler, 1995	28 (♂)	13+X_1X_2	this study
Arctosa sp.	28 (♂)	13+X_1X_2	Mittal, 1960
	30 (♀)		Mittal, 1963
Tricca lutetiana (Simon, 1876)	28 (♂)	13+X_1X_2	this study
Xerolycosa miniata (C.L. Koch, 1834)	22 (♂)	10+X_1X_2	Hackman, 1948
	22 (♂)	10+X_1X_2	Gorlov et al., 1995
	22 (♂)	10+X_1X_2	this study
Xerolycosa nemoralis (Westring, 1861)	26 (♂)	12+X_1X_2	Hackman, 1948
	22 (♂)	10+X_1X_2	Gorlov et al., 1995
	22 (♂)	10+X_1X_2	this study

unknown or uncertain (Table 1). Up to now, 102 species of lycosids (including 23 species determined only to the genus level) from 21 genera have been analysed. These data were recently summarised by Král & Buchar (1999) and Chemisquy et al. (2008); the list of the latter authors did not include six species studied by Yang et al. (1997), Bugayong et al. (1999), Akan et al. (2005), and Kumbiçak et al. (2009).

Diploid chromosome numbers of lycosid males range from 2n = 18 in *Lycosa* sp. (Srivastava & Shukla, 1986) to 2n = 28 in most other species. Suzuki (1954) classified spider karyotypes into three types based on the number of chromosomes: (i) karyotypes with high chromosome numbers (2n > 46 in males) are regarded as primitive (mesothelids, mygalomorphs), (ii) those with 2n = 34–46 (the so-called intermediate type), and (iii) those with low chromosome numbers (2n < 34). From this point of view, karyotypes of lycosids are of type iii, which is the most frequent type of karyotype recorded for spiders. C-values of the lycosid genome (1.83–4.18 pg, mean 2.65 pg) fall within the range of that recorded for entelegyne spiders (0.74–5.7 pg) (Gregory & Shorthouse, 2003).

Lycosids have a "typical" entelegyne karyotype that is composed exclusively of acrocentric chromosomes (Rowell, 1990). C-banding revealed that on the chromosomes of lycosids there is a centromeric block of constitutive heterochromatin (CH) (Table 2). Despite the uniform distribution of CH in the karyotype, particular species can differ considerably in the proportion of AT- and CG-rich heterochromatin (Chemisquy et al., 2008). The distribution of nucleolar organizer regions (NORs) has only been recorded for *Allocosa georgicola* (Walckenaer, 1837). Wise (1983) found that nucleoli are associated only with two autosome pairs in this species.

Most species of lycosids retain the original sex chromosome system of spiders, ♂X_1X_2/♀$X_1X_1X_2X_2$ (the so-called X_1X_20 system) (Suzuki, 1954; Datta & Chatterjee, 1989). In some species the sex chromosomes are the largest elements of the complement, e.g. as in *Lycosa erythrognatha* Lucas, 1836 (2n = 22) (Chemisquy et al., 2008). In *Hippasa madhuae* Tikader & Malhotra, 1980 (2n = 28), the sex chromosomes are medium-sized (Parida et al., 1986). *Schizocosa malitiosa* (Tullgren, 1905) (2n = 22) is reported to be polytypic for the size of the sex chromosomes (Chemisquy et al., 2008). In lycosid spiders, the X_2 chromosome is usually only slightly shorter than X_1. However, both sex chromosomes can differ considerably in size. For example, in *Pardosa astrigera* L. Koch, 1878 and *Pirata piratoides* (Bösenberg & Strand, 1906), the X_1 is the longest chromosome of the karyotype and X_2 the shortest (Yang et al., 1996, 1997). Only in six species are there derived systems. The ♂X0/♀XX system is reported in four wolf spiders: *Lycosa nordenskjoldi* Tullgren, 1905 (Diaz & Saez, 1966), *Schizocosa* sp. of the *malitiosa* group (Postiglioni & Brum-Zorrilla, 1981), *Lycosa barnesi* Gravely, 1924, and *Wadicosa quadrifera* (Gravely, 1924) (Srivastava & Shukla, 1986). In *Lycosa* sp. of the *thorelli* group the ♂$X_1X_2X_3$/♀$X_1X_1X_2X_2X_3X_3$ system is found (Postiglioni & Brum-Zorrilla, 1981). An analysis of two closely related species of the *thorelli* group indicates that this system evolved from the X_1X_20 system by non-disjunction (Postiglioni & Brum-Zorrilla, 1981). Finally, translocations between autosomes and sex chromosomes occur as polymorphism in a population in *Pardosa morosa* (L. Koch, 1870) (J. Král, unpubl.).

As in other spiders, the behaviour of X chromosomes of wolf spiders during male meiosis is peculiar. By

TABLE 2. Localization of the constitutive heterochromatin (CH) and nucleolar organizer regions (NORs) of the lycosids studied.

Species	Marker studied	Localization	References
Allocosa georgicola (Walckenaer, 1837)	NORs	Associated with two pairs of autosomes	Wise, 1983 (sub. *Lycosa georgicola*)
Alopecosa albofasciata (Brullé, 1832)	CH	Medium sized blocks, pericentromeric location	Gorlova et al., 1997
Arctosa alpigena lamperti Dahl, 1908	CH NORs	Medium sized blocks, pericentromeric location Third and eleventh pairs – distal location	this study
Lycosa erythrognatha Lucas, 1836	CH	Medium sized blocks, pericentromeric location	Chemisquy et al., 2008
Lycosa thorelli (Keyserling, 1877)	CH	Medium sized blocks, pericentromeric location	Brum-Zorrilla & Postiglioni, 1980
Lycosa sp.	CH	Medium sized blocks, pericentromeric location	Brum-Zorrilla & Postiglioni, 1980
Pardosa astrigera L. Koch, 1878	CH	Medium sized blocks, pericentromeric location	Yang et al., 1996
Pirata piraticus (Clerck, 1757)	CH	Medium sized blocks, pericentromeric location	Peng et al., 1998
Pirata piratoides (Bösenberg & Strand, 1906)	CH	Medium sized blocks, pericentromeric location	Yang et al., 1997
Schizocosa malitiosa (Tullgren, 1905)	CH	Medium sized blocks, pericentromeric location	Brum-Zorrilla & Cazenave, 1974 (sub. *Lycosa malitiosa*)
Tricca lutetiana (Simon, 1876)	CH NORs	Tiny blocks, pericentromeric location Eighth and eleventh pairs – distal location	this study
Xerolycosa miniata (C.L. Koch, 1834)	CH NORs	Tiny blocks, pericentromeric location Third and sixth pairs – distal location	this study
Xerolycosa nemoralis (Westring, 1861)	CH NORs	Tiny blocks, pericentromeric location Third and sixth pairs – distal location	this study

prophase I they are located at the periphery of the nucleus, pair non-homologously without chiasma formation and segregate to one of the spindle poles as a single unit during anaphase I (e.g. Hackman, 1948). Furthermore, the male X chromosomes are more condensed than autosomes and thus stain more intensively (so-called positive heteropycnosis) during some meiotic stages, especially prophase I. Structures ensuring the pairing of non-homologous X chromosomes in *Schizocosa malitiosa* are visible when viewed under a transmission electron microscope. This revealed that X_1 and X_2 are connected by an unusual structure, designated the "junction lamina", from pachytene to diplotene (Benavente & Wettstein, 1977). The junction lamina resembles the synaptonemal complex from which it differs in having a discoidal shape and indistinct central part.

The present study focused on the central European members of *Arctosa*, *Tricca*, and *Xerolycosa*. The diploid chromosome numbers, chromosome morphology, sex chromosome system, distribution of CH and NORs were determined and compared with those recorded for related species in the literature.

MATERIAL AND METHODS

Spiders were collected either by hand or by using dry pitfall traps. If necessary, they were reared to a stage suitable for analysis. Detailed data on the specimens examined are presented in Table 3. It was found that subadult (penultimate) and/or adult males are the most suitable for this type of analysis. Their testes contained spermatogonial mitoses and various stages of meiosis. Female mitotic metaphases were obtained from the intestine. Selected specimens are deposited in the collection of the National Museum, Prague (numbers P6A-4927–P6A-4931).

Suitable tissues were removed from the opisthosoma using fine scissors and placed in a physiological solution, developed for *Ephestia* Guenée, 1845 (Lockwood, 1961), on a wax plate. Chromosomes were prepared using a spreading technique (Traut 1976), with the following modifications: the gonads were hypotonized in 0.075 M KCl for 12–15 min at room temperature (RT) and fixed in two changes of freshly prepared ethanol : acetic acid p.a. (3 : 1) (standard preparations) or methanol : acetic acid p.a. (3 : 1) (standard preparations, preparations for silver staining and C-banding) for 10 and 20 min (RT). Using a pair of fine tungsten needles a cell suspension was prepared quickly and as finely as possible from a piece of tissue in a drop of 60% acetic acid on a clean slide. The preparation was placed on a histological plate heated to 40°C (standard preparations) or 33–34°C (to preserve the chromatin for silver staining or C-banding). The cell suspension was moved by pushing it with a tungsten needle until the drop had almost evaporated. The remaining suspension was discarded. Preparations were stained the next day with 5% Giemsa solution (Merck, Darmstadt, Germany) in Sörensen buffer (pH 6.8) for 27–28 min (RT).

The standard method of Sumner (1972) was used for C-banding. Preparations were stained with 5% Giemsa solution in Sörensen buffer (pH 6.8) for 70 min (RT). Staining of NORs by $AgNO_3$ was performed according to Howell & Black (1980), with some modifications to standardise the method for lycosid chromosomes. Three drops of gelatine and seven drops of $AgNO_3$ were dripped on the preparation (as the viscosity of these reagents differ the drops of gelatine are bigger than those of $AgNO_3$) and mixed gently using a glass stick. The slide was covered by a cover slip, placed on a histological plate (50–51°C) covered by four-layer cellulose cotton wool and incubated (4–5 min) until solution was the colour of onion skin. Finally, the cover slide was removed under a gentle current of tap water and the preparation air-dried.

Preparations were inspected under a Jenaval (Carl Zeiss) or Olympus BX 50 microscope (Olympus). Selected plates were photographed using an immersion lens 100×. The images were

TABLE 3. List of species studied.

Species	Locality	Co-ordinates	Individuals studied	Date(s) of experiment
Arctosa alpigena	Sportgastein (A), alpine meadow (cca 2050 m), Kreuzkogel mountain, 20.vii.2008 (L.K. lgt., P.D. det.)	47°03′N, 13°05′E	1♀	30.iv.2009
Arctosa alpigena lamperti	Modrava (CZ), locality "Zadní mlynářská slať" near the ford of the Roklanský potok brook	49°01′N, 13°27′E	4s♂	vii.2007, viii.2007, vi.2008 *
Arctosa cinerea	Tušť near Suchdol nad Lužnicí (CZ)	48°54′N, 14°54′E	1♂	21.v.2004
Arctosa figurata	Srbsko (CZ); locality "Komárkova lesostep", the Dřínová hora mountain, 11.vi.2009 (P.D. lgt. et det.)	49°57′N, 14°10′E	2♂	12.vi.2009
Arctosa leopardus	Žleby (CZ); sandy warp, Doubrava river, 1.v.2009 (P.D. lgt., J.B. det.)	49°53′N, 15°29′E	1♂	14.v.2009; collected as a s and reared until adult moult (10.v.2009) #
Arctosa maculata	Žilina near Nový Jičín (CZ), bank of Jičínka river	49°35′N, 18°02′E	1♂	1.ix.1995
Arctosa perita	Mušlov near Mikulov (CZ)	48°47′N, 16°41′E	3s♂	20.x.2003
Arctosa renidescens	Patsch (A), Patscherkofel mountain	47°12′N, 11°27′E	4s♂	8.ix.2000
Tricca lutetiana	Srbsko (CZ); locality "Komárkova lesostep", the Dřínová hora mountain (P.D. lgt. et det.)	49°57′N, 14°10′E	6s♂	xi.2005, viii.2006, v.2007 #
			4♂	vi.2005, vi.2006, ix.2006
Xerolycosa miniata	Brandýs nad Labem (CZ)	50°10′N, 14°39′E	5s♂	v.2005, xi.2005
			1♂	13.v.2005
	Tušť near Suchdol nad Lužnicí (CZ)	48°54′N, 14°54′E	9s♂	28.v.2006
			3♂	28.v.2006
	Praha – Hloubětín (CZ)	50°07′N, 14°32′E	4s♂	vi.2006, ix.2006
Xerolycosa nemoralis	Těptín (CZ)	49°53′N, 14°35′E	7s♂	vi.2006, viii.2006, ix.2006
			10♂	vi.2006, x.2006

A – Austria, CZ – Czech Republic, s – subadult, * the best stage for studying spermatogonial mitosis seems to be one week after moulting to the subadult stage, whereas for studying meiosis it is two weeks after this moult; # subadult males were suitable for obtaining spermatogonial mitoses, complete meioses occur in males up to one week after the final moult.

recorded on a film (Kodak Technical Pan or Kodak Ilford Panf Plus 50) or by using a digital camera DP71 (Olympus). Well spread spermatogonial metaphases or sister metaphases II were used for karyotype analysis. The measurements were carried out using Image Tool 3 (UHTSCA, University of Texas Health Science Center at San Antonio, TX, USA) programme. Karyotypes were constructed in Corel Photo-Paint X3 (Version 13) software. Relative chromosome lengths were calculated as a percentage of the total chromosome length of the diploid set, including the sex chromosomes (% TCL). The X_2/X_1 ratio was calculated from ten spermatogonial metaphases and ten diplotene stages. Chromosome morphology was classified according to Levan et al. (1964). The mean chiasma frequency was calculated using the well spread complete diplotene plates for each species and dividing the total number of chiasmata by total number of bivalents.

RESULTS

Arctosa C.L. Koch, 1847

Karyotype and spermatogonial mitosis

The karyotypes consisted of 28 chromosomes (Fig. 1A–E). All chromosomes were acrocentric, except one subtelocentric autosome pair (No. 3) in *A. alpigena* and *A. a. lamperti* (Fig. 1D). The length of autosome pairs decreased gradually (Table 4), except in *A. figurata* and *A. perita* (Fig. 1C). The first autosome pairs of these species were notably larger than the other autosome pairs. At mitotic metaphase, chromosomes were usually arranged radially. On the sixth chromosome pair in *A. leopardus* there was a distinct subterminal secondary constriction (Fig. 1B). In *A. a. lamperti*, the secondary constriction on the third autosome pair was adjacent to centromeric block of heterochromatin (Fig. 1D). The sex chromosome system was of the ♂X_1X_2/♀$X_1X_1X_2X_2$ type. The two X chromosomes were of unequal length (Table 4). The length of chromosome X_2 varied from 66.48% (*A. cinerea*) to 94.10% (*A. renidescens*) of the length of chromosome X_1. In *A. cinerea* (Fig. 1A), *A. a. lamperti* and *A. maculata*, the X_1 at spermatogonial mitoses was the longest chromosome of the set. The X_2 was usually the second longest chromosome (Table 4). In *A. cinerea* and *A. leopardus*, its length ranged between that of the second and third autosome pair. *Arctosa perita* is exceptional as the length of the X_1 lay between those of the seventh and eighth autosome pair, and X_2 between the eleventh and twelfth pair. Sex chromosomes exhibited a slight positive heteropycnosis at metaphase in some species (*A. cinerea*, *A. a. lamperti*, *A. leopardus*, and *A. perita*). Moreover, one (*A. cinerea*) or both (*A. perita*) sex chromosomes

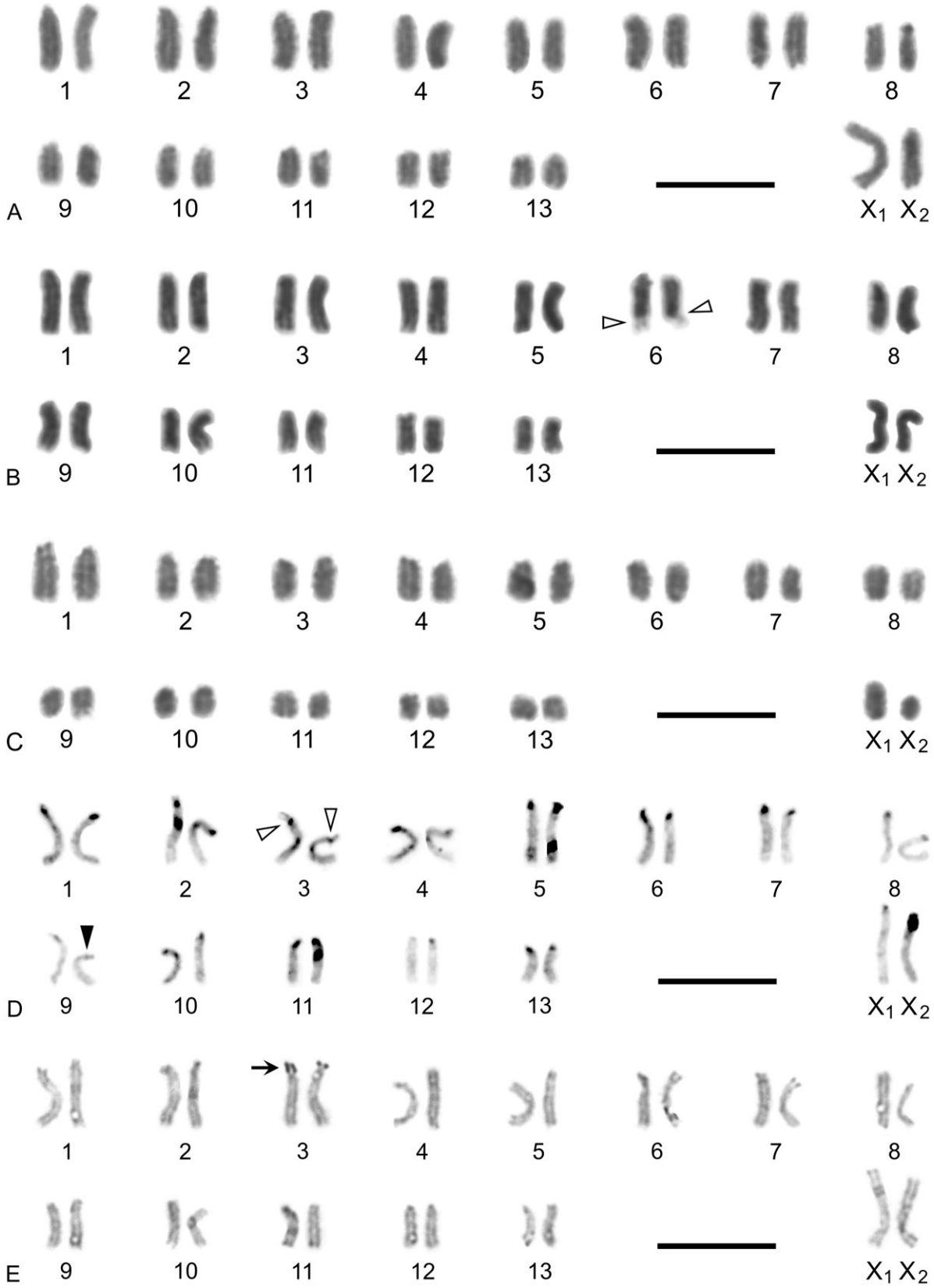

Fig. 1. A–E: Male karyotypes of *Arctosa* (2n♂ = 28, X_1X_20) (based on spermatogonial metaphases). A – *Arctosa cinerea*. B – *Arctosa leopardus*. There are subterminal secondary constrictions (white arrowheads) on the sixth pair of autosomes. C – *Arctosa perita*, note relatively short sex chromosomes and difference in their size. D – *Arctosa alpigena lamperti*, C-banded figure. Note prominent blocks of centromeric CH on all chromosomes, a secondary constriction on the third pair of autosomes (white arrowheads), and a double block of proximal CH on one chromosome of pair No. 9 (black arrowhead). E – *Arctosa alpigena lamperti*, silver stained figure. There is an NOR at the proximal ends of the third pair and distal ends of the eleventh pair. One NOR is duplicated (arrow). Bars = 10 μm.

TABLE 4. Relative chromosome lengths of every pair of autosomes and sex chromosomes of the species of *Arctosa*, *Tricca*, and *Xerolycosa* studied. For comparing male autosomes and gonosomes note that the relative length of one gonosome is half that of an appropriate pair of autosomes. X_2/X_1 – size ratio of chromosomes X_2 and X_1. (M – spermatogonial metaphase, D – diplotene). Chiasma frequency, numbers in parenthesis – number of diplotenes studied in order to determine the chiasma frequency.

Genus	Species	Sex	Autosome pair No.													Sex chromosomes				Chiasma frequency (D)
			1	2	3	4	5	6	7	8	9	10	11	12	13	X_1	X_2	X_2/X_1 (M) (%)	X_2/X_1 (D) (%)	
Arctosa	*A. cinerea*	♂	9.76	8.91	8.35	7.92	7.71	7.50	7.11	6.37	5.81	5.40	5.07	4.70	4.22	6.69	4.45	66.48	71.05	1.008 (10)
	A. figurata	♂	10.04	8.11	7.80	7.74	7.59	7.23	6.95	6.53	5.98	5.87	5.46	5.26	4.77	6.28	4.39		79.56	1.000 (17)
	A. leopardus	♂	8.91	8.38	7.97	7.84	7.76	7.48	7.24	7.00	6.73	6.25	5.71	5.20	4.68	4.74	4.09	86.09	70.75	1.007 (52)
	A. maculata	♂	8.99	8.49	8.43	7.93	7.68	7.45	6.90	6.59	6.24	5.83	5.44	5.14	6.67	5.45	4.70	86.38	78.77	
	A. perita	♂	10.93	9.71	8.78	8.37	8.05	7.58	7.17	6.68	6.08	5.76	5.25	4.99	4.62	3.46	2.58	77.17	71.60	1.000 (10)
Arctosa alpigena-group	*A. alpigena*	♀	8.26	7.97	7.59	7.55	7.04	6.78	6.02	5.85	5.62	5.44	5.05	4.93	4.32	9.13	8.45	92.55		
	A. alpigena lamperti	♂	8.68	8.32	8.13	7.86	7.62	7.32	6.97	6.68	6.25	6.07	5.82	5.74	5.10	4.92	4.53	92.05	83.12	1.004 (173)
	A. renidescens	♂	9.15	8.74	8.29	8.05	7.57	7.33	6.99	6.74	6.18	6.03	5.62	5.24	4.87	4.73	4.45	94.10		1.004 (20)
Tricca	*T. lutetiana*	♂	9.14	8.56	8.33	8.06	7.64	7.32	6.96	6.61	6.12	5.80	5.48	5.11	4.56	5.36	4.94	92.66	88.66	1.002 (301)
Xerolycosa	*X. miniata*	♂	11.36	10.92	10.15	9.66	9.33	8.96	8.69	8.58	8.15	7.54				3.58	3.07	86.69	78.44	1.000 (15)
	X. nemoralis	♂	11.36	10.44	10.10	9.81	9.45	9.16	8.96	8.42	8.21	7.52				3.44	3.13	90.62	84.87	1.019 (37)

exhibit slightly delayed separation of chromatids at metaphase. In other species, this phenomenon was not observed.

The pattern of CH and NORs was studied in *A. a. lamperti.* Metaphase chromosomes showed relatively large centromeric CH blocks (Fig. 1D). One chromosome of the ninth autosome pair bore a double block of CH proximally (Fig. 1D). The X_1 chromosome had a relatively small amount of centromeric CH compared with X_2 and autosomes. Two autosome pairs had terminal NORs. Length measurements indicate that the NORs are on the third (proximal position) and eleventh (distal position) pair of chromosomes (Fig. 1E). Duplication of the NOR on one chromosome of the third pair was visible on several plates of one specimen (Fig. 1E).

Male meiosis

Sex chromosomes manifested positive heteropycnosis from leptotene (Fig. 2A) and zygotene to diakinesis (*A. a. lamperti*, *A. maculata*, *A. renidescens*), anaphase I (except metaphase I) (*A. figurata*), or metaphase II (except prophase II) (*A. cinerea*, *A. leopardus*, *A. perita*). By zygotene (Fig. 2B), the autosome bivalents showed a distinct polarization forming the typical bouquet persisting till pachytene (Fig. 2C). By pachytene, chromatin of each bivalent was differentiated into distinct chromomeres. Proximal ends of bivalents showed prominent centromeric knobs (Fig. 2C). In *A. a. lamperti*, the third-largest bivalent contained a secondary constriction (Fig. 2D). Sex chromosomes formed a body situated at the periphery of the nucleus (Fig. 2D). In hypotonized pachytene nuclei of females, sex chromosome bivalents were indistinguishable (Fig. 2C).

The diplotene stage is relatively long. Diplotene plates consisted of 13 bivalents. Sex chromosomes persisted in the form of a body till early diplotene (most species) (Fig. 3A) or late diplotene (*A. a. lamperti*) (Fig. 3B). After that, the sex chromosome body split up into particular sex chromosomes that were closely aligned but not associated. The sex univalents in late diplotene were either as long as the medium-sized autosome bivalents (*A. figurata*, *A. maculata*, *A. renidescens*) (Fig. 3A) or nearly as short as the shortest autosome bivalent (*A. a. lamperti*, *A. cinerea*, *A. leopardus*, *A. perita*) (Fig. 3B). Both sex chromosomes differed in size (Table 4). The length of chromosome X_2 varied from 70.75% (*A. leopardus*) to 83.12% (*A. a. lamperti*) of the length of chromosome X_1. Some plates contained one or two bivalents with two chiasmata (one proximal and one distal) (Table 4). In contrast to the other species, bivalents of *A. figurata* and *A. perita* had always only one (usually proximal) chiasma.

The so-called terminalization of chiasmata (seeming shift of chiasmata to the ends of bivalents due to spiralization of chromosomes) occurred at diakinesis (Fig. 3C) in *Arctosa* as well as in the following genera. At metaphase I, all bivalents segregated at the same time (Fig. 3D). By anaphase I, the two X chromosomes behaved as a unit and moved together to the same pole. This resulted in two types of anaphase I plates. At this stage, the sister chromatids of autosomes were associated only by their centromeres and thus had a V-shaped appearance, whereas chromatids of sex chromosomes had not sepa-

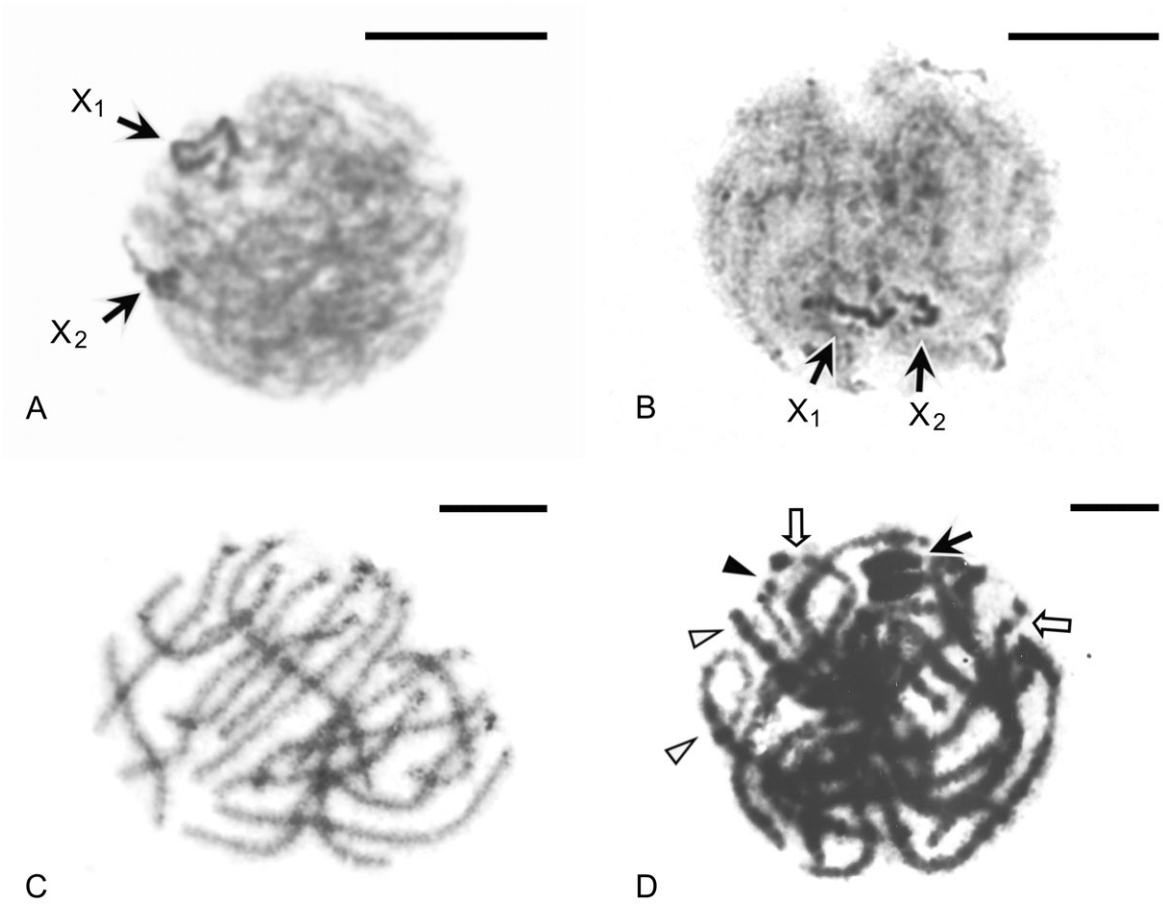

Fig. 2. A–D: *Arctosa*, early prophase I. A – *Arctosa leopardus* (♂), leptotene, sex chromosomes are located at the periphery of the nucleus (arrows). B – *Arctosa perita* (♂), late zygotene, bivalents form bouquet, sex chromosomes are heteropycnotic (arrows). C – *Arctosa alpigena* (♀), pachytene consists of 15 bivalents forming a bouquet, note centromeric knobs at proximal ends of bivalents. D – *Arctosa alpigena lamperti* (♂), pachytene, sex chromosomes are aligned in parallel (black arrow) at the periphery of the nucleus. Note interchromomeres (white arrowheads), terminal knobs (black arrowhead) and subterminal secondary constrictions (white arrows). Bars = 10 μm.

rated (Fig. 3E). The chromosomes were comparatively larger than at metaphase I due to some decondensation of chromatin. Two types of metaphases II were observed: one with 15 elements (13 autosomes and X_1X_2) and the other with only 13 autosomes (Fig. 3F). The sex chromosomes exhibited slightly delayed separation of chromatids. In *A. a. lamperti*, the chromatids of all chromosomes were coiled at metaphase II (Fig. 3F).

***Tricca lutetiana* (Simon, 1876)**

Spermatogonial mitosis

The mitotic plates consisted of 28 acrocentric chromosomes (Fig. 4A). Length of autosome pairs decreased gradually (Table 4). Third-largest autosome pair had a distinct subterminal secondary constriction. The sex chromosome system was ♂X_1X_2/♀$X_1X_1X_2X_2$. The two X chromosomes were the longest chromosomes of the karyotype, but they differed in size (Table 4). However, they were morphologically indistinguishable from the autosomes. Metaphase chromosomes had a minute centromeric CH block (Fig. 4A). Longer chromosomes had a usually somewhat larger amount of CH than the shorter ones, the CH of which was hardly visible. Two autosome pairs (the eighth and eleventh) possessed a distal NOR (Fig. 4B).

Male meiosis

By leptotene, sex chromosomes formed two intensely stained structures. Sex chromosomes retained positive heteropycnosis up to early diplotene. By zygotene, bivalents were arranged in the typical bouquet. At zygotene and pachytene, the sex chromosomes were situated at the periphery of the nucleus forming a heterochromatinic body both in standard and C-banded preparations (Fig. 4C). Chromatin of pachytene bivalents formed distinct chromomeres. At early diplotene (Fig. 5A) 13 autosome bivalents and a body formed by two sex chromosomes were observed. However, at late diplotene (Fig. 5B), the sex chromosome body suddenly decondensed into two rod-like chromosomes, which were as long as the longest autosome bivalent. Both sex univalents differed in size (Table 4). During this period, they were isopycnotic with the autosomes (Fig. 5B). An achromatic decondensed region appeared on each sex univalent approximately in the proximal third or quarter (Fig. 5B, C). The position of the constriction differed between both sex univalents

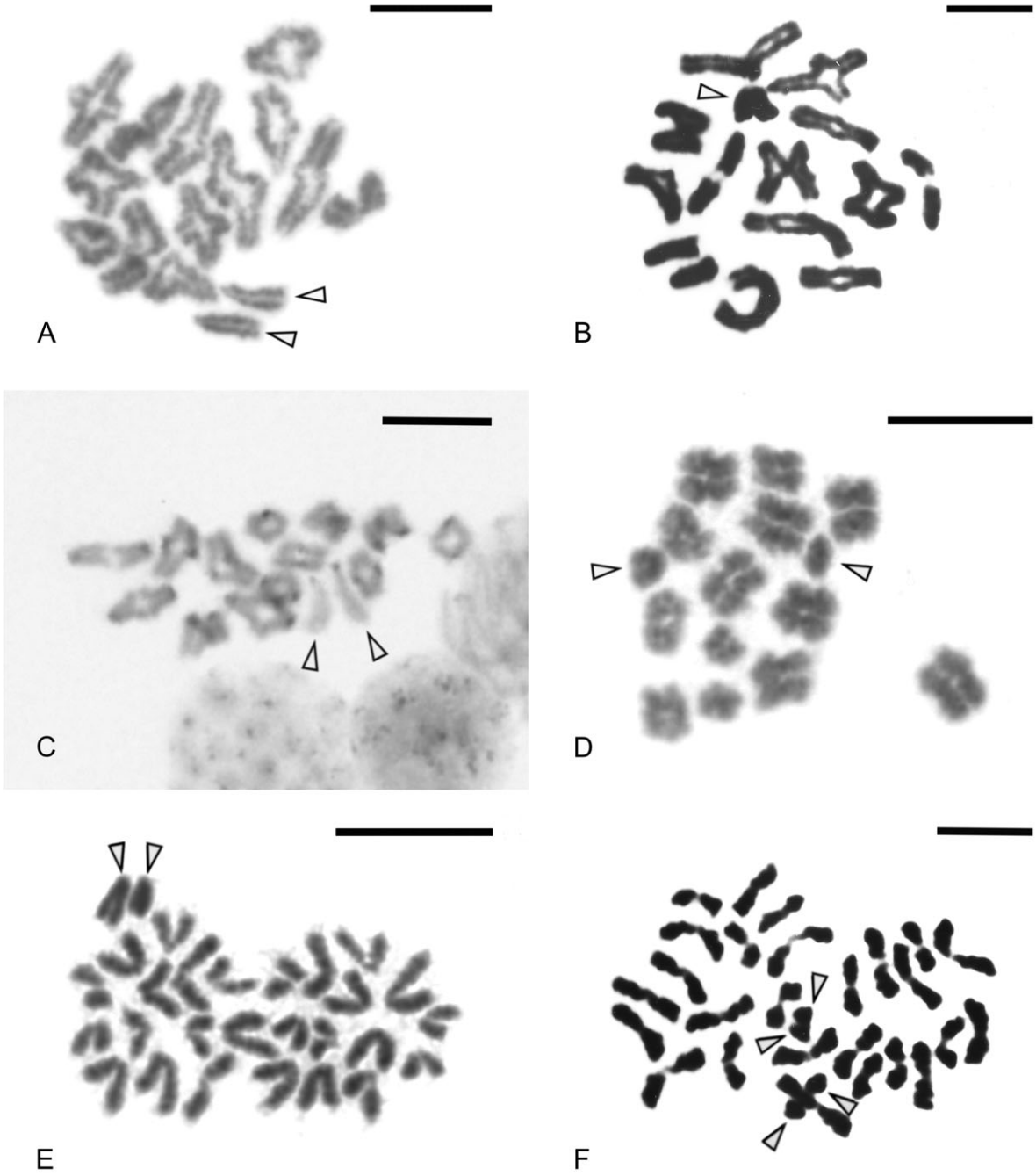

Fig. 3. A–F: *Arctosa* (2n♂ = 28, X_1X_20), male meiosis, continued. Arrowheads indicate sex chromosomes. A – *Arctosa figurata*, diplotene, the sex univalents are arranged in parallel. B – *Arctosa alpigena lamperti*, diplotene, the X univalents are paired proximally. C – *Arctosa alpigena lamperti*, C-banded diakinesis. The centromeric regions of the X univalents, which are formed of constitutive heterochromatin, converge. D – *Arctosa cinerea*, metaphase I. E – *Arctosa cinerea*, anaphase I. F – *Arctosa alpigena lamperti*, two sister metaphase II, chromosomes are coiled to form macrocoils (Ohnuki coils). Bars = 10 μm.

(Fig. 5B). In most cases, each bivalent had one (usually proximal) chiasma (Table 4, Fig. 5A–D). The sex chromosomes recondensed at diakinesis (Fig. 5D) and remained loosely associated at the periphery of the nucleus. The course of following meiotic stages does not differ from those of *Arctosa*.

Xerolycosa Dahl, 1908

Spermatogonial mitosis

The mitotic plates contained 22 chromosomes (Fig. 6A). All chromosomes were acrocentric and decreased gradually in size (Table 4). They were usually arranged radially at metaphase. The sex chromosome system was X_1X_20. The two gonosomes were the shortest chromosomes; however, they were of different size (Table 4). CH and NORs were studied in both species. Chromosomes

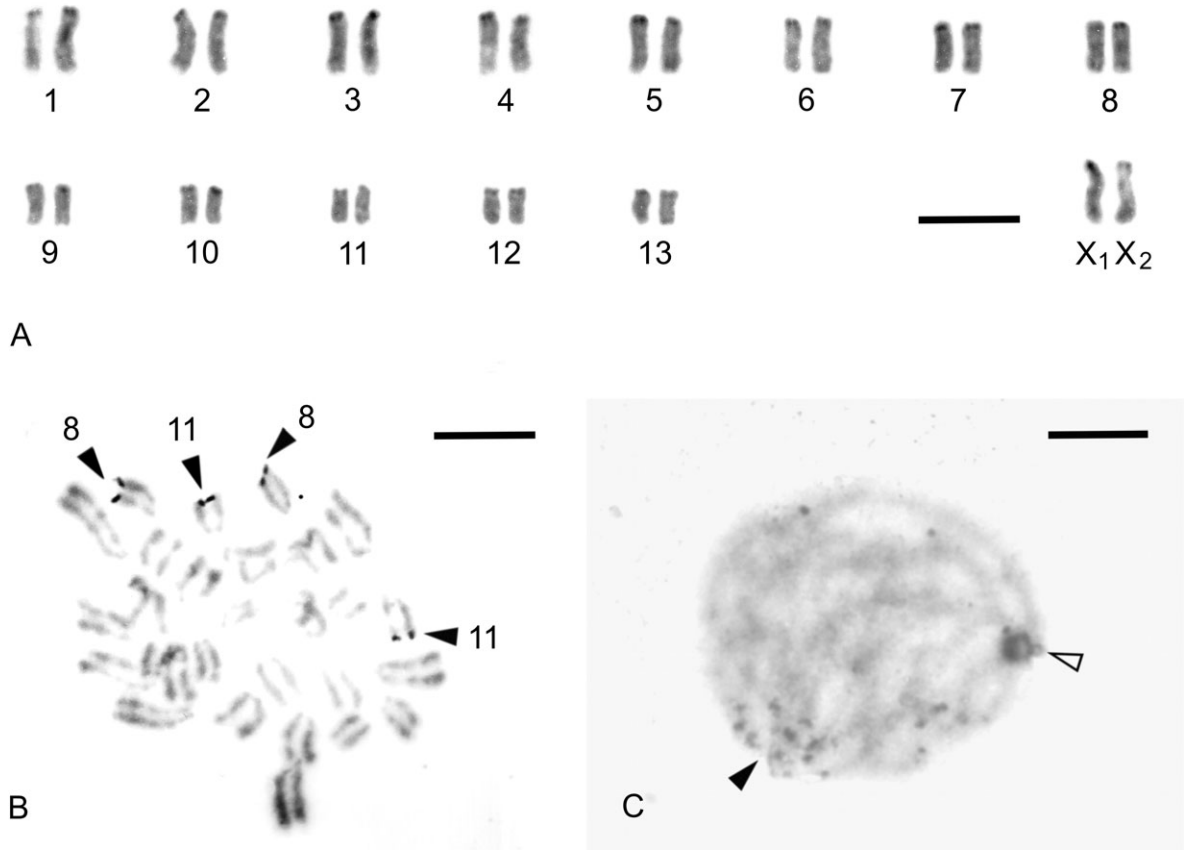

Fig. 4. A–C: Male of *Tricca lutetiana* (2n♂ = 28, X_1X_20). A – karyotype (based on a C-banded spermatogonial metaphase). Chromosomes contain a low amount of CH, which is located in the centromeric regions. B – silver-stained late mitotic metaphase. Note there are NORs at the distal ends of autosome pairs No. 8 and 11 (black arrowheads). C – C-banded pachytene. Note completely heterochromatinized sex body (white arrowhead). Due to arrangement of autosome bivalents into bouquet, most pericentromeric blocks of heterochromatin form a cluster at the periphery of the nucleus (black arrowhead). Bars = 10 μm.

showed minute, not clearly recognisable, centromeric CH blocks (Fig. 6B). Two pairs of autosomes (the third and sixth pair) had distal NORs (Fig. 6C). The duplication of NORs on one chromosome of the third pair was seen on several plates of both species (Fig. 6C).

Male meiosis

During leptotene, sex chromosomes formed two positively heteropycnotic structures. The pattern of sex chromosome heteropycnosis was different in both species. In *X. nemoralis*, heteropycnosis disappeared by early diplotene. In contrast, heteropycnosis persisted until anaphase I in *X. miniata*. The course of zygotene and pachytene was similar to that in the genera *Arctosa* and *Tricca*. At pachytene, positively heteropycnotic sex chromosomes formed a sex body, which was situated at the periphery of the nucleus.

During diplotene in *X. miniata* (Fig. 7A), ten autosome bivalents and two sex chromosomes were closely associated with each other. The sex chromosomes differed in size (Table 4). They started to separate at early diplotene and were always nearly as short as the shortest autosome bivalent. Each bivalent had only one (usually proximal) chiasma. At diakinesis (Fig. 7B), the sex univalents were situated in parallel at the periphery of the nucleus.

At early diplotene in *X. nemoralis* (Fig. 7C), ten autosome bivalents and a body formed by sex chromosomes were observed. The sex chromosomes were the shortest chromosomes. However, at late diplotene (Fig. 7D), the sex univalents decondensed suddenly forming two parallel rods, which were as long as the longest autosome bivalent. Proximal parts of sex chromosomes were achromatic and separated by a conspicuous constriction. At this stage, the sex chromosomes were isopycnotic with the autosomes (Fig. 7D). In most cases, each autosome bivalent had one (usually proximal) chiasma. Occasionally, one to three pairs formed ring bivalents with proximal and distal chiasmata (Table 4). In early diakinesis, the sex chromosomes appear isopycnotic with autosomes; their proximal constrictions were still visible (Fig. 7E). The sex chromosomes re-condensed at late diakinesis (Fig. 7F).

During anaphase I (Fig. 8A) in both *Xerolycosa* species, the segregation of autosomes and sex chromosomes was analogous to those described in *Arctosa*. At metaphase II (Fig. 8B), two types of spermatocytes were present, namely with twelve (ten autosomes and X_1X_2) and ten chromosomes (ten autosomes). The sex chromosomes of *X. miniata* exhibit slightly delayed separation of chromatids (Fig. 8B).

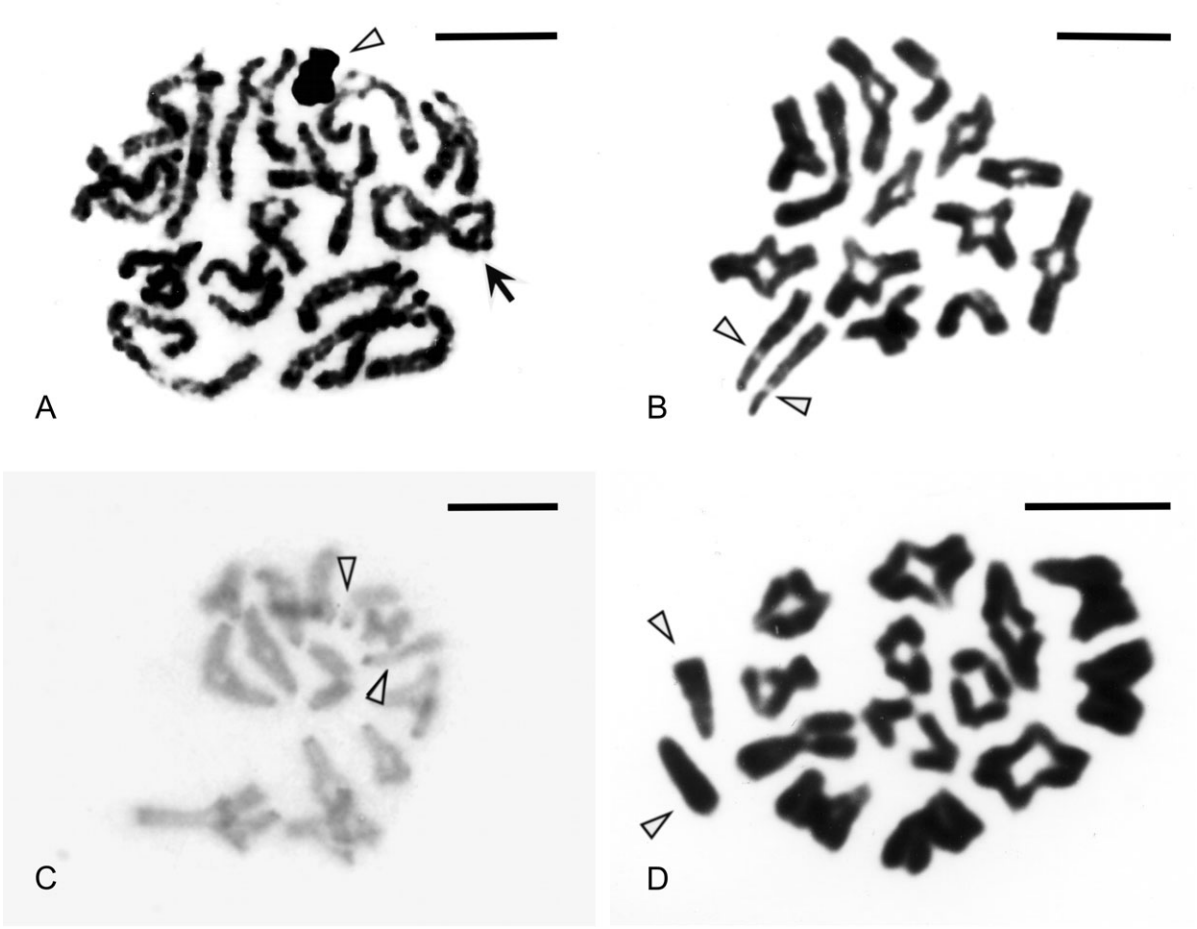

Fig. 5. A–D: *Tricca lutetiana* (2n♂ = 28, X_1X_20), male prophase I. A – the sex body (arrowhead) is located at the periphery of the nucleus in early diplotene. Note a bivalent with two chiasmata (arrow). B – sex chromosomes are aligned in parallel and partially decondensed, with achromatic regions at their proximal ends (arrowheads) in late diplotene. C – C-banded diplotene, sex chromosomes have achromatic regions at their proximal ends (arrowheads). D – diakinesis, sex chromosomes (arrowheads) are recondensed and loosely associated. Bars = 10 μm.

DISCUSSION AND CONCLUSIONS

Present study provides basic karyotype data for eleven central European lycosid spiders of the genera *Arctosa*, *Tricca* and *Xerolycosa*. The former two genera are placed by Petrunkevitch (1928) in the subfamily Lycosinae, the latter genus belongs, according to Zyuzin (1985), to Evippinae. Six species were studied for the first time and the karyotype data for another five species revised (Table 1). The chromosome number recorded for *X. nemoralis* corresponds with that reported for Siberian populations studied by Gorlov et al. (1995) but differs from that reported for Finish spiders by Hackman (1948). The data presented here for *A. alpigena* and *A. leopardus* also differ from that reported by Hackman (1948). Furthermore, the diploid number for *A. perita* recorded in this study is incongruent with that reported from Turkey by Akan et al. (2005). Misidentification of spiders by these authors is unlikely as these species are relatively easy to identify. The discrepancies may be due to either interpopulation variability in chromosome numbers or the chromosome numbers cited by these authors are incorrect. As interspecific variability in chromosome numbers in entelegyne spiders is in general very low (Brum-Zorrilla & Postiglioni, 1980; Král, 1994; Chen, 1999) the latter possibility seems to be the most likely. The techniques currently used to prepare chromosomes often produce incomplete plates, which was also the case in the present study. Moreover, the method of chromosome preparation used by Hackman (1948) did not include treatment with a hypotonic solution so the spreading of chromosomes is likely to have been less pronounced than in the present study. Therefore, that author probably failed to determine the correct chromosome number of *A. alpigena*, *A. leopardus* and *X. nemoralis*. Results provided by Akan et al. (2005) for *A. perita* are also problematic. The published chromosome plates are of very low quality and not suitable for determining chromosome numbers. The reported 2n for females (2n = 12) is improbable as the lowest diploid number recorded in lycosids so far is 2n = 18 in a male of *Lycosa* sp. from India (Srivastava & Shukla, 1986). Similarly, the results for other species presented in that study are also improbable. The authors record 2n = 16 for a female of the theraphosid spider, *Chaetopelma olivaceum* (C.L. Koch, 1841) (sub. *C. anatolicum* Schmidt & Smith, 1995), whereas unpublished results (J.

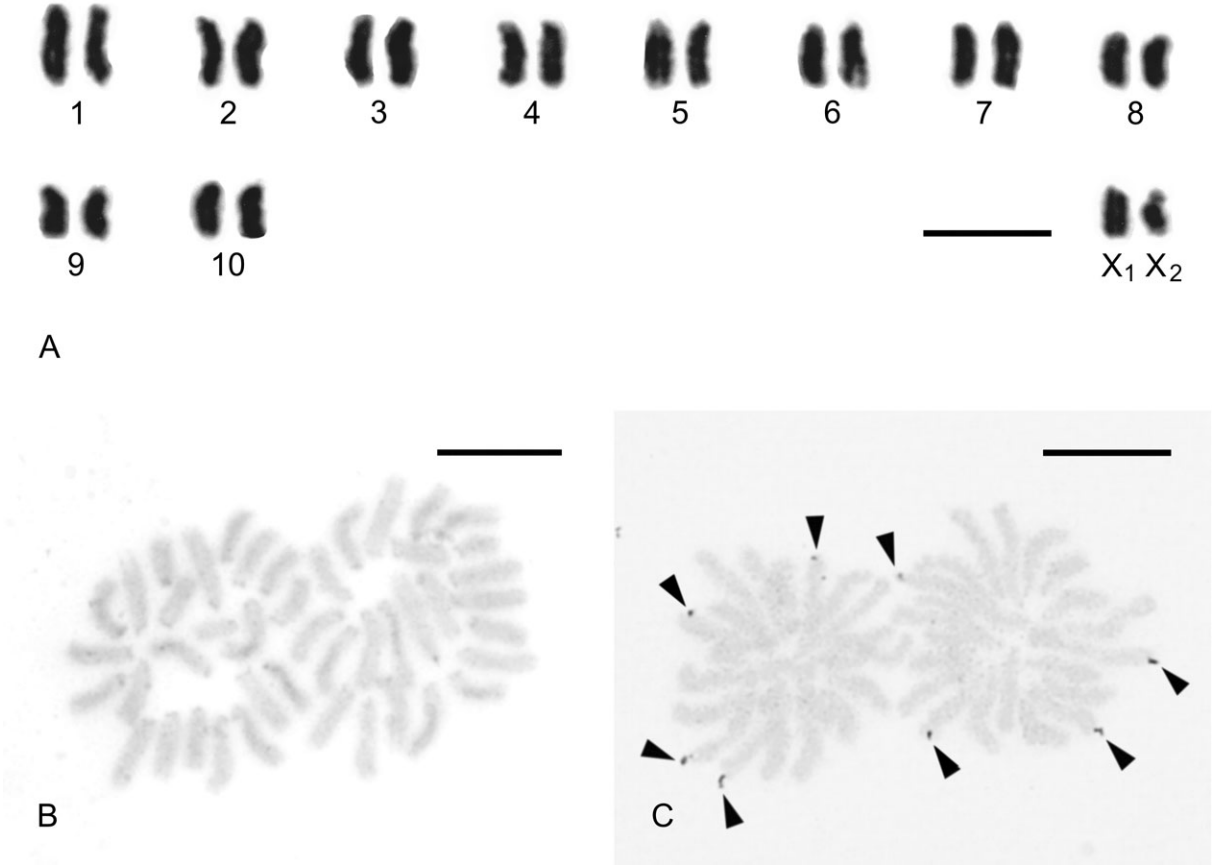

Fig. 6. A–C: Mitotic metaphase of *Xerolycosa* (2n♂ = 22, X_1X_20). A – *Xerolycosa nemoralis*, karyotype. B – *Xerolycosa nemoralis*, C-banding, there is a negligible amount of CH in centromeric regions of chromosomes. C – *Xerolycosa miniata*, NORs present at distal ends of two pairs of autosomes (arrowheads). Bars = 10 μm.

Král, unpubl.) indicate a much higher 2n for species of this genus (> 60 chromosomes).

Currently there are karyotypes recorded for 108 species of lycosid (Chemisquy et al., 2008; Kumbiçak et al., 2009; this study). Male karyotype of 28 chromosomes is found in 56% of the species, 2n = 22 in 18%, 2n = 26 in 12% and 2n = 24 in 8%. Other diploid chromosome numbers (2n = 27, 23, 20, 19 and 18) are recorded for only one or two species (see Chemisquy et al., 2008 for a review). The results presented here support the idea of previous authors (Sharma et al., 1958; Datta & Chatterjee, 1989; Král & Buchar, 1999) that the ancestral karyotype of lycosid males consists of 28 acrocentric chromosomes. This chromosome number was recorded in this study for all central European species of the genera *Arctosa* and *Tricca*. Based on the compilation of Král & Buchar (1999) and Giroti et al. (2005, 2007) it is suggested that 2n = 28 is also the ancestral karyotype of the related families Trechaleidae, Pisauridae, Oxyopidae, Ctenidae, and Senoculidae. Hence it could be the ancestral karyotype for the whole superfamily Lycosoidea sensu lato (sensu Jocqué & Dippenaar-Schoeman, 2007).

During the evolution of some lycosid lineages, diploid chromosome numbers were gradually reduced, as in other entelegyne spiders, most probably by tandem fusions (Suzuki, 1954). Decrease by cycles of Robertsonian translocations and subsequent pericentric inversions suggested by White (1973) is less probable because karyotypes of lycosids do not contain biarmed (i.e. metacentric or submetacentric) autosome pair(s). Reduced chromosome numbers were found in almost all the lycosid subfamilies examined (Evippinae, Lycosinae, Pardosinae, Venoniinae s. lat. including Piratinae and Wadicosinae) in addition to the ancestral number of 2n = 28 (see Chemisquy et al., 2008 for review). A tendency towards reduced chromosome numbers is particularly obvious in the subfamilies Lycosinae, Evippinae, and Venoniinae s. lat. as is evident from the derived karyotypes of both species of *Xerolycosa* (2n = 22). Heterogeneity of diploid chromosome numbers in the subfamily Lycosinae is due to variability of 2n in the genus *Lycosa* (18–28 chromosomes). However, the variability in *Lycosa* reflects the absence of a modern taxonomic revision of this genus rather than a tendency towards a reduction in 2n. Therefore, considerable reductions in 2n most probably only occur in the latter two subfamilies. Interestingly, molecular data (Murphy et al., 2006) place these subfamilies in a monophyletic clade. Such congruence between karyotype and molecular data is interesting.

The sex chromosome system found in all species is of the ♂X_1X_2/♀$X_1X_1X_2X_2$ type, as in the majority of wolf spiders studied (see Chemisquy et al., 2008 for review).

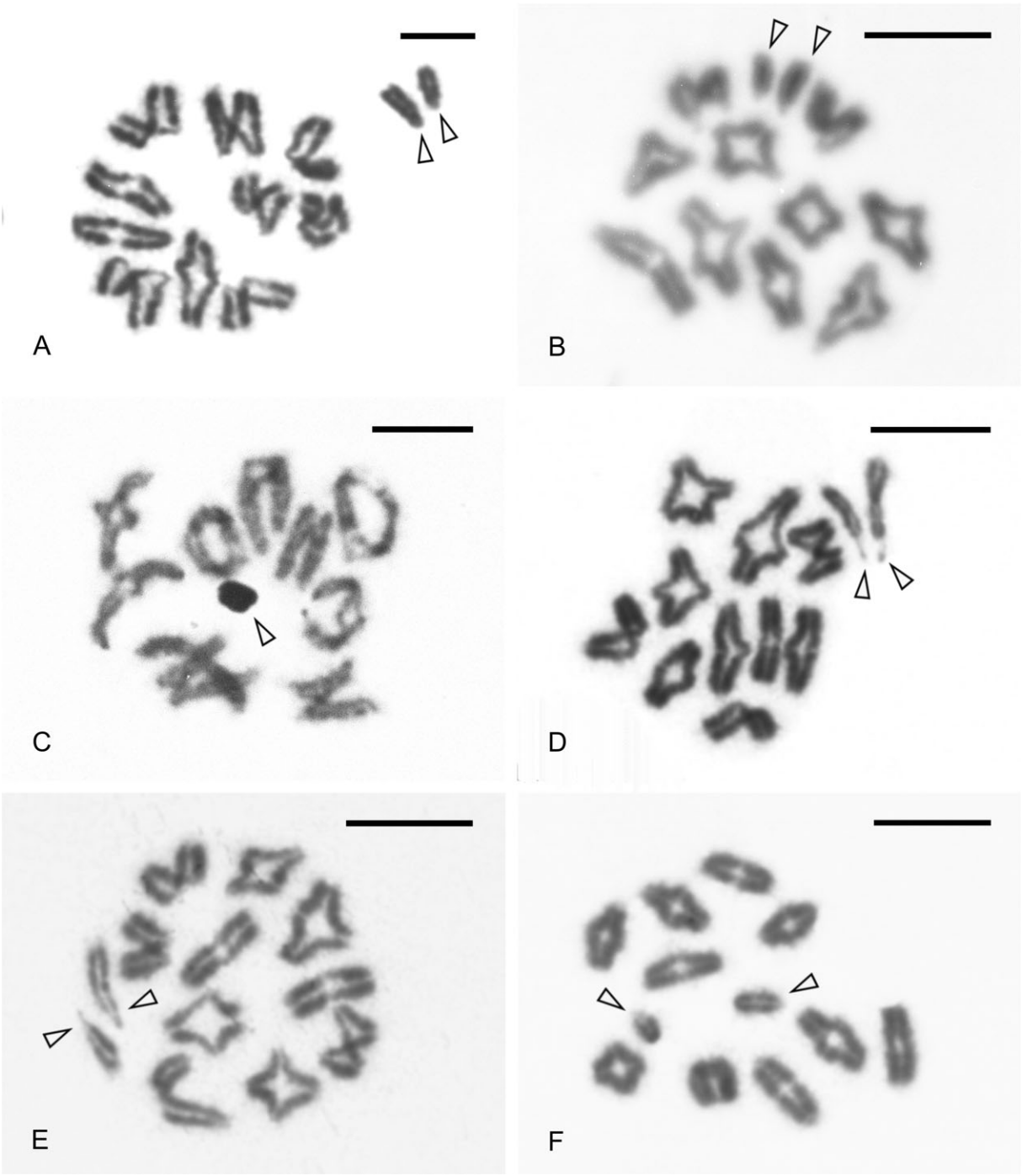

Fig. 7. A–F: *Xerolycosa* (2n♂ = 22, X_1X_20), male prophase I. Arrowheads – sex chromosomes. A, B: *Xerolycosa miniata*. A – sex chromosomes are aligned at the periphery of the nucleus at late diplotene. B – the sex chromosomes are aligned in parallel at early diakinesis. C–F: *Xerolycosa nemoralis*. C – sex chromosomes form a body at early diplotene. Note one ring bivalent. D – sex chromosomes are partially decondensed at late diplotene. Proximal regions of the sex chromosomes are achromatic. E – sex chromosomes are still partially decondensed at early diakinesis. F – sex chromosomes are recondensed and separate at late diakinesis. Bars = 10 μm.

This system is thought to be the ancestral type for spiders (Suzuki, 1954) including Lycosidae (Datta & Chatterjee, 1989). In all species examined, sex chromosomes were unequal in length. The length difference of X_1 and X_2 chromosome was usually smaller at diplotene than in spermatogonial metaphases, possibly a consequence of more intensive condensation of sex chromosomes at diplotene. Interestingly, the difference in the length of the X_1 and X_2 chromosome is often species-specific and less or more variable among related species (Table 4). Differences in the size of the sex chromosomes could reflect evolutionary changes in the amount of sex chromosome heterochromatin. The quantity of CH and its distribution often changes quickly during evolution (King, 1993).

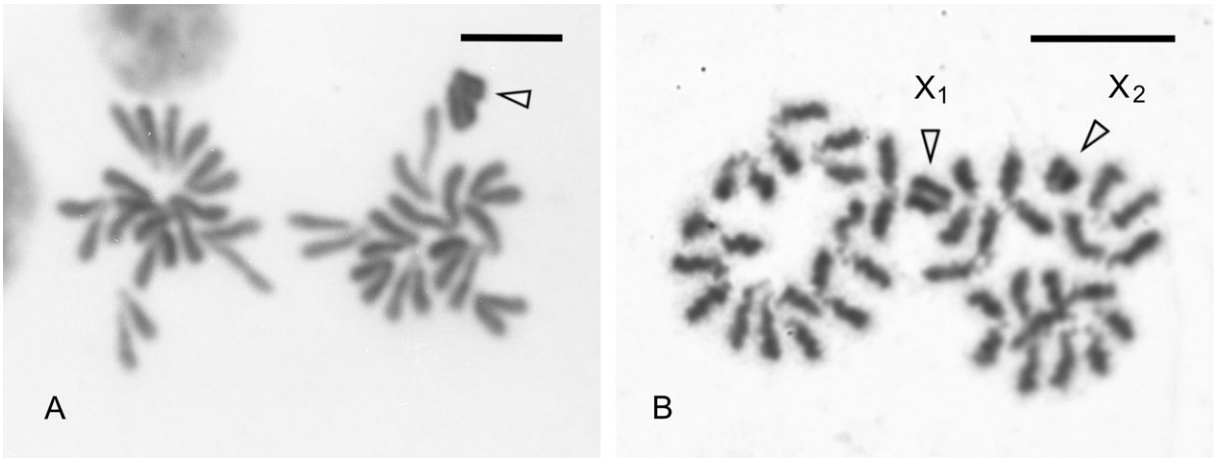

Fig. 8. A–B: *Xerolycosa miniata* (2n♂ = 22, X_1X_20), male meiosis, continued. A – heteropycnotic sex chromosomes (arrowhead) are close together at anaphase I. B – at metaphase II the heteropycnotic sex chromosomes (with their chromatids not yet separate) are at the periphery of the right plate (arrowheads). Bars = 10 μm.

However, the heterochromatin content of the sex chromosomes of the lycosids studied is too low to account for the relatively large change in the X_2/X_1 ratio, even between related species. Therefore, it is assumed that differences in the sizes of sex chromosomes can reflect also the rearrangement of sex chromosomes, especially deletions (Suzuki, 1952) or translocations between sex chromosomes. The sex chromosomes were the two longest chromosomes in the karyotype of most species. In *A. cinerea* and *A. leopardus*, the X_1 was the longest chromosome while the length of X_2 ranged between that of the second and third autosomal pair. In *A. perita*, the length of X_1 was between that of the seventh and eighth autosomal pair, and X_2 between the eleventh and twelfth pair. In both *Xerolycosa* species, the two gonosomes were the shortest chromosomes. These differences may be a consequence of the rearrangements of sex chromosomes and/or autosomes, which resulted in changes in their size, most probably due to translocations. The occurrence of autosomal translocations is indicated also by the fact that the first pair of autosomes of two *Arctosa* species (*A. perita* and *A. figurata*) was notably larger than the other pairs of autosomes, whereas in all the other species of *Arctosa* the pairs of autosomes gradually decreased in size. Translocations could result in the development of barriers to reproduction between species (King, 1993) and therefore, could affect karyotype evolution in the genus *Arctosa*.

Distribution of CH in spiders has been studied only in representatives of several families of both basal araneomorphs (Král et al., 2006) and entelegyne araneomorphs. In entelegyne spiders, CH is recorded in some species of the families Salticidae (Gorlova et al., 1997), Tetragnathidae (Araújo et al., 2005), Sparassidae (Rowell, 1985; Rodríguez-Gil et al., 2007), Agelenidae (Král, 2007), and Lycosidae (this work, Table 2). Entelegyne spiders have centromeric CH and sometimes a tiny telomeric block on several chromosomes. Only centromeric CH, without detectable distal blocks, is known for lycosids (see Table 2 for references). This pattern in the distribution of CH is believed to be the ancestral type for spiders (Rodríguez-Gil et al., 2007). Genera *Arctosa*, *Tricca*, and *Xerolycosa* have only centromeric CH. In wolf spiders studied previously (Table 2) the blocks of CH are relatively large. In this study relatively large blocks were found also in *A. a. lamperti*. In contrast to this, the blocks in the remaining three species (*T. lutetiana* and both *Xerolycosa* species) were very small. The sex chromosomes in the species investigated had the same distribution and amount of heterochromatin as the autosomes. According to the data presented, sex chromosomes of wolf spiders consist mostly of euchromatin and are heterochromatinized facultatively at male meiosis as in other spiders (Datta & Chatterjee, 1989; Král, 2007; Rodríguez-Gil et al., 2007). Predominance of CH in sex chromosomes has been reported only in a few spiders, namely three species of lycosids. Thus, X chromosomes were found to be formed only of CH at spermatogonial metaphases in *Lycosa* sp. (Brum-Zorrilla & Postiglioni, 1980) and male diplotene in *Alopecosa albofasciata* (Gorlova et al., 1997). Similarly, one sex chromosome of female *Schizocosa malitiosa* was suggested to be formed only of CH at mitotic metaphase (Brum-Zorrilla & Cazenave, 1974). The results presented indicate that C-banded sex chromosomes were completely heterochromatinized in some nuclei at the zygotene and pachytene stages of meiosis. During the later stages of meiosis and mitosis, sex chromosomes had the standard distribution of heterochromatin blocks. Therefore, complete heterochromatinization of C-banded sex chromosomes at the onset of meiosis reflected the fact that they were very condensed rather than predominance of CH. In this manner, it is possible to explain also complete heterochromatinization of sex chromosomes after application of C-banding in above mentioned species, especially heterochromatinization of meiotic sex chromosomes reported by Gorlova et al. (1997).

In the present study, silver-staining was used for the first time to detect NORs of wolf spiders. The location of NORs has been recorded only for some basal araneomorph (Král et al., 2006; Oliveira et al., 2007; Araujo et

al., 2008) and entelegyne spiders (Wise, 1983; Král, 1995; Araújo et al., 2005; Rodríguez-Gil et al., 2007). Unlike in the entelegynes, NORs of basal araneomorphs are often situated on sex chromosomes (Král et al., 2006). In all the lycosids studied (Table 2), two autosome pairs have an NOR. This distribution and number of NORs are reported for representatives of three unrelated families of entelegyne spiders, Nephilidae (Araújo et al., 2005), Sparassidae (Rodríguez-Gil et al., 2007), and Lycosidae (this study). Therefore, this pattern could be ancestral one for entelegyne spiders.

In *T. lutetiana*, the pairs of chromosomes No. 8 and 11 have distal NORs. *Arctosa a. lamperti* has a different pattern of NORs. The NOR on chromosome pair No. 11 is located at the distal end whereas that on subtelocentric chromosome pair No. 3 is located at the proximal end. Despite the little information on the position of NORs in spiders, they appear to be rarely proximally located. This situation is reported only for one autosome pair of two basal araneomorphs, *Diguetia albolineata* (O.P.-Cambridge, 1895) (Diguetidae) and *Monoblemma muchmorei* Shear, 1978 (Tetrablemmidae) (Král et al., 2006). Therefore, the pericentromeric position of NOR in *A. a. lamperti* is probably a derived character. This NOR may have been translocated onto the third pair from a former location on the eighth pair, which is its position in the closely related genus *Tricca*. There are no pairs of subtelocentric autosomes in other species of *Arctosa* (except *A. alpigena*) so that pericentromeric NORs could be restricted only to species closely related to *A. a. lamperti.* Therefore, it would be worth examining the evolution of NORs in the remaining species of the "*alpigena*-group".

Interestingly, the duplication of NORs was found on one chromosome pair of *A. a. lamperti* and *Xerolycosa* species. This duplication probably occurred in the heterozygous state, as it was only found on one chromosome of the pair. NOR duplications seem to be common in entelegyne spiders; they are reported also in representatives of the families Agelenidae (Král, 1995, 2007) and Nephilidae (Araújo et al., 2005).

Similar to other lycosids the genera analysed exhibit a low chiasma frequency during meiosis. By diplotene, most bivalents have only one chiasma. In general, a low chiasma frequency is typical for acrocentric chromosomes (e.g. John, 1990). Although the course of meiosis is the same in all the lycosid genera studied here, the particular species differ considerably in many details of sex chromosome behaviour, some of which appeared as species-specific characters. The most remarkable is the behaviour of the sex chromosomes in *T. lutetiana* and *X. nemoralis* at late prophase I. Sex chromosomes of these species exhibit partial decondensation and are isopycnotic with autosome bivalents at late diplotene, whereas those of the other species are condensed and exhibit positive heteropycnosis. During that period, proximal parts of sex chromosomes of *T. lutetiana* bear curious achromatic regions. In order to consider the taxonomical significance of this feature, it would be necessary to examine the meiotic behaviour of sex chromosomes in taxa potentially related to *Tricca* (e.g. the genus *Triccosta* Roewer, 1960 and *Arctosa* species that were included in the former genera *Leaena* Simon, 1885 and *Leaenella* Roewer, 1960). The occurrence of constrictions on the X chromosomes of *X. nemoralis* at diplotene could also have some taxonomic importance because they appear in *X. nemoralis* but not in *X. miniata*. Therefore, the question is whether they occur also in other *Xerolycosa* species. Concerning the constrictions, it would be worth examining the meiotic sex chromosomes in males of the genera potentially related to *Xerolycosa* (e.g. the genus *Evippa* Simon, 1885).

Although karyotypes of *Arctosa* species are all characterized by the same diploid number, chromosome morphology and sex chromosome system, they differ in many details. The same was found in the genus *Xerolycosa*. In view of the low karyotype diversity in both genera, their cytotaxonomy is likely to be best resolved using molecular cytogenetic methods that are suitable for studying the evolution of conservative karyotypes.

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